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| (54) Title: RECOMBINANT CYTOMEGALOVIRUS VACCINE (57) Abstract The present invention provides a non-defective adenovirus recombinant expression system for the expression of an immunogenic fragment of the HCMV gB subunit, said recombinant HCMV-expressing adenovirus being useful as a vaccine. | | |

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RECOMBINANT CYTOMEGALOVIRUS VACCINE

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HD-18957. The U.S. government has certain rights in this
invention.

Field of the Invention

10 The present invention refers generally to a
recombinant human cytomegalovirus vaccine, and more
specifically to a subunit vaccine containing fragments of
a HCMV major glycoprotein complex subunit gB gene.

15 Background of the Invention

Cytomegalovirus (CMV) is one of a group of
highly host specific herpes viruses that produce unique
large cells bearing intranuclear inclusions. The
envelope of the human cytomegalovirus (HCMV) is
20 characterized by a major glycoprotein complex recently
termed gB or gCI, which was previously referred to as gA.
HCMV causes cytomegalic inclusion disease and has been
associated with a syndrome resembling infectious
mononucleosis in adults. It also induces complications
25 in immunocompromised individuals.

CMV infection in utero is an important cause of
central nervous system damage in newborns. Although the
virus is widely distributed in the population, about 40%
of women enter pregnancy without antibodies and thus are
30 susceptible to infection. About 1% of these women
undergo primary infection in utero. Classical
cytomegalic inclusion disease is rare; however, a
proportion of the infected infants, including those who
were symptom-free, are subsequently found to be mentally
35 retarded.

Preliminary estimates based on surveys of approximately 4,000 newborns from several geographical areas indicate that the virus causes significant damage of the central nervous system leading to mental deficiency in at least 10%, and perhaps as high as 25%, of infected infants. Assuming that about 1% of newborn infants per year excrete CMV and that about one fourth of those develop mental deficiency, in the United States this means approximately 10,000 brain-damaged children born per year. This is a formidable number, particularly in view of the ability of these children to survive [J. Infect. Dis., 123 (5):555 (1971)].

HCMV in humans has also been observed to cause serious complications and infections in the course of organ transplantations, especially with kidney and liver transplants.

Several HCMV vaccines have been developed or are in the process of development. Vaccines based on live attenuated strains of HCMV have been described. [See, e.g., S. A. Plotkin et al, Lancet, 1:528-30 (1984); S. A. Plotkin et al, J. Infect. Dis., 134:470-75 (1976); S. A. Plotkin et al, "Prevention of Cytomegalovirus Disease by Towne Strain Live Attenuated Vaccine", in Birth Defects, Original Article Series, 20(1):271-287 (1984); J. P. Glazer et al, Ann. Intern. Med., 91:676-83 (1979); and U. S. Patent 3,959,466.] A proposed HCMV vaccine using a recombinant vaccinia virus expressing HCMV glycoprotein B has also been described. [See, e.g., Cranage, M. P. et al, EMBO J., 5:3057-3063 (1986).] However, vaccinia models for vaccine delivery are believed to cause local reactions. Additionally, vaccinia vaccines are considered possible causes of encephalitis.

Adenoviruses have been developed previously as efficient heterologous gene expression vectors. For example, an adenovirus vector has been employed to express herpes simplex virus glycoprotein gB [D. C. Johnson et al, Virology, 164:1-14 (1988)]; human immunodeficiency virus type 1 envelope protein [R. L. Dewar et al, J. Virology, 63:129-136 (1988)]; and hepatitis B surface antigen [A. R. Davis et al, Proc. Natl. Acad. Sci., U.S.A., 82:7560-7564 (1985); J. E. Morin et al, Proc. Natl. Acad. Sci., U.S.A., 84:4626-4630 (1987)]. Adenoviruses have also been found to be non-toxic as vaccine components in humans [See, e.g., E. T. Takajuki et al, J. Infect. Dis., 140:48-53 (1970); P. B. Collis et al, J. Inf. Dis., 128:74-750 (1973); and R. B. Couch et al, Am. Rev. Respir. Dis., 88:394-403 (1963)].

There remains a need in the art for additional vaccines capable of preventing CMV infection by generating neutralizing antibody and cellular responses to CMV in the human immune system.

Summary of the Invention

In one aspect, the present invention provides a non-defective recombinant adenovirus containing a fragment of a gB subunit of the HCMV free from association with any additional human proteinaceous material. In this recombinant adenovirus, the HCMV subunit is under the control of regulatory sequences capable of expressing the HCMV gB subunit fragment *in vitro* and *in vivo*.

Another aspect of the present invention is a vaccine composition comprising a non-defective recombinant adenovirus, as described above.

In a further aspect, the invention provides a method of vaccinating a human against HCMV comprising administering to the patient the recombinant adenovirus

containing the subunit gene encoding a gB protein fragment in a vaccine composition. The inventors have found that this method of presenting these HCMV gene fragments to a vaccinate is particularly capable of eliciting an immune response.

In still a further aspect the invention provides an adenovirus-produced gB subunit fragment, which fragment may also form vaccine compositions to protect humans against HCMV. Currently, the preferred fragment comprises about amino acids 1 to about 303 of the gB protein SEQ ID NO:2, gB₁₋₃₀₃.

Other aspects and advantages of the present invention are described further in the following detailed description of preferred embodiments of the present invention.

Brief Description of the Drawings

Fig. 1A illustrates diagrammatically the cloning of the gB gene into the early region 3 (E3) transcription unit of Ad5. Represented are the 3.1kb fragment containing the gB gene by the open box; the adenovirus sequences extending from 59.5 to 100 mu (except for the deletion of the 78.5 to 84.7 mu length) by the filled portion of the circle; the large BamHI fragment of the pBR322 by the thin line of the circle. In the figure, the restriction enzymes are identified as follows: X is XbaI, B is BamHI.

Fig. 1B illustrates diagrammatically the construction of the recombinant adenovirus virus Ad5/gB, containing the gB gene of the Towne strain of HCMV described in Example 1. This figure shows the 59.5 mu to 76 mu region where homologous recombination occurs (as indicated by the crossed lines) between wild type Ad5 viral sequence and the adenovirus sequences present on the pAd5 plasmid containing the gB gene. The plaque

purified recombinant virus retains the cloning XbaI sites and the direction of transcription of the gB gene from the E3 promoter is indicated by the bent arrow. Restriction enzymes are as identified above.

5

Detailed Description of the Invention

The present invention provides novel immunogenic components for HCMV which comprise an adenovirus expression system capable of expressing a
10 selected HCMV subunit gene fragment *in vivo*. Alternatively the selected subunit fragment for use in an immunogenic composition, such as a vaccine, may be expressed in, and isolated from, the recombinant adenovirus expression system.

15 As provided by the present invention, any adenovirus strain capable of replicating in mammalian cells *in vitro* may be used to construct an expression vector for the selected HCMV subunit. However, a preferred expression system involves a non-defective
20 adenovirus strain, including, but not limited to, adenovirus type 5. Alternatively, other desirable adenovirus strains may be employed which are capable of being orally administered, for use in expressing the CMV subunit *in vivo*. Such strains useful for *in vivo*
25 production of the subunit in addition to adenovirus-5 strains include adenovirus type 4, 7, and 21 strains. [See, e.g., Takajuji et al, cited above]. Appropriate strains of adenovirus, including those identified above and those employed in the examples below are publicly
30 available from sources such as the American Type Culture Collection, Rockville, Maryland.

Similarly, a number of strains of isolated human CMV may be employed from which a desired gB subunit is derived. For example, the Towne strain of CMV, a
35 preferred strain for use in preparation of a vaccine of

this invention because of its broad antigenic spectrum and its attenuation, was isolated from the urine of a two month old male infant with cytomegalic inclusion disease (symptoms - central nervous system damage and
5 hepatosplenomegaly). This strain of CMV was isolated by Stanley A. Plotkin, M.D. and is described in J. Virol., 11 (6): 991 (1973). This strain is freely available from The Wistar Institute or from the ATCC under accession number VR-977. However, other strains of CMV useful in
10 the practice of this invention may be obtained from depositories like the ATCC or from other institutes or universities.

In the practice of one embodiment of this invention the HCMV subunit may be produced *in vitro* by
15 recombinant techniques in large quantities sufficient for use in an immunogenic composition or subunit vaccine. Alternatively, the recombinant adenovirus containing the subunit may itself be employed as an immunogenic or vaccine component, capable of expressing the subunit *in*
20 *vivo*.

The presently preferred subunit proteins for use in the present invention are the HCMV gB subunit fragments. One embodiment of the present invention provides a replication competent (non-defective)
25 adenovirus vector carrying a fragment of the HCMV gB gene which contains a CTL epitope and/or B cell epitope. A preferred gene fragment encodes about amino acid 1 to about amino acid 303 of the gB subunit protein SEQ ID NO:2. Another suitable fragment of gB SEQ ID NO:2 is the
30 fragment spanning about amino acid 1 to about amino acid 700 of SEQ ID NO:2. Still another suitable gB fragment spans about amino acid 1 to about amino acid 465 of SEQ ID NO:2.

More particularly, it is anticipated that
35 smaller fragments containing all or a portion of the gB

fragment spanning amino acids about 155 to about 303 will also be desirable for vaccine use. This region is suspected of containing at least a CTL epitope (see Examples 5 and 6 below).

5 It is anticipated that in the construction of the adenovirus vectors of this invention, any of the subunits of the HCMV envelope protein may be employed. In a manner similar to the use of the gB fragment in this vaccine, other subunits of CMV which may be employed in
10 the production of a vaccine according to the invention may be selected from the gcII, gcIII, or immediate early subunits of the human virus. Alternatively, more than one HCMV subunit may be employed in a vaccine according to the teachings of the present invention.

15 In addition to isolating the desired subunit from an available strain of HCMV for insertion into the selected adenovirus, the sequences of the subunits of two HCMV strains have been published [See, e.g., Mach et al, J. Gen. Virol., 67:1461-1467 (1986); Cranage et al,
20 (1986) cited above; and Spaete et al, Virol., 167:207-225 (1987). These subunit sequences can therefore be chemically synthesized by conventional methods known to one of skill in the art, or the sequences purchased from commercial sources.

25 The recombinant adenovirus of the present invention may also contain multiple copies of the HCMV subunit. Alternatively, the recombinant virus may contain more than one HCMV subunit type, so that the virus may express two or more HCMV subunits, subunit
30 fragments, or immediate early antigens and subunits together.

 In the construction of the adenovirus vector of the present invention, the CMV subunit sequence is preferably inserted in an adenovirus strain under the
35 control of an expression control sequence in the virus

itself. The adenovirus vector of the present invention preferably contains other sequences of interest in addition to the HCMV subunit. Such sequences may include regulatory sequences, enhancers, suitable promoters, secretory signal sequences and the like. The techniques employed to insert the subunit sequence into the adenovirus vector and make other alterations in the viral DNA, e.g., to insert linker sequences and the like, are known to one of skill in the art. See, e.g., T. Maniatis et al, "Molecular Cloning. A Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982). Thus, given the disclosures contained herein the construction of suitable adenovirus expression vectors for expression of an HCMV subunit protein is within the skill of the art. Example 3 below provides construction details for the non-defective adenovirus containing these gB fragments.

The recombinant adenovirus itself, constructed as described above, may be used directly as an immunogen or a vaccine component. According to this embodiment of the invention, the recombinant adenovirus, containing the HCMV subunit, e.g., the gB subunit fragment, is introduced directly into the patient by vaccination. The recombinant virus, when introduced into a patient directly, infects the patient's cells and produces the CMV subunit in the patient's cells. The inventors have found that this method of presenting these HCMV genes to a vaccinee is particularly capable of eliciting an immune response. Examples 5 and 6 demonstrate the ability of a recombinant adenovirus containing the gB fragment, amino acid 1-303 of SEQ ID NO:2, to induce a gB-specific, protective CTL response in mice.

The use of these adenovirus recombinants as immunogens capable of inducing a CTL response is surprising in view of the results obtained in the same

assays of the examples with other known virus types, which have been used in vaccines previously. According to another embodiment of this invention, once the recombinant viral vector containing the CMV subunit protein, e.g., the gB₁₋₃₀₃ subunit fragment, is
5 constructed, it may be infected into a suitable host cell for *in vitro* expression. The infection of the recombinant viral vector is performed in a conventional manner. [See, Maniatis et al, supra.] Suitable host
10 cells include, without limitation, mammalian cells and cell lines, e.g., A549 (human lung carcinoma) or 293 (transformed human embryonic kidney) cells.

The host cell, once infected with the recombinant virus of the present invention, is then
15 cultured in a suitable medium, such as Minimal Essential Medium (MEM) for mammalian cells. The culture conditions are conventional for the host cell and allow the subunit, e.g., gB₁₋₃₀₃ subunit fragment, to be produced either intracellularly, or secreted extracellularly into the
20 medium. Conventional protein isolation techniques are employed to isolate the expressed subunit from the selected host cell or medium.

When expressed *in vitro* and isolated from culture, the subunit, e.g., gB₁₋₃₀₃, may then be formulated
25 into an appropriate vaccine composition. Such compositions may generally contain one or more of the recombinant CMV subunits.

The preparation of a pharmaceutically acceptable vaccine composition, having appropriate pH,
30 isotonicity, stability and other conventional characteristics is within the skill of the art. Thus such vaccines may optionally contain other components, such as adjuvants and/or carriers, e.g., aqueous suspensions of aluminum and magnesium hydroxides.

Thus, the present invention also includes a method of vaccinating humans against human CMV infection with the recombinant adenovirus vaccine composition. This vaccine composition is preferably orally administered, because adenoviruses are known to replicate in cells of the stomach. Previous studies with adenoviruses have shown them to be safe when administered orally [see, e.g., Collis et al, cited above]. However, the present invention is not limited by the route of administration selected for the vaccine.

When the recombinant adenovirus is administered as the vaccine, a dosage of between 10^5 and 10^8 plaque forming units may be used. Additional doses of the vaccines of this invention may also be administered where considered desirable by the physician. The dosage regimen involved in the method for vaccination against CMV infection with the recombinant virus of the present invention can be determined considering various clinical and environmental factors known to affect vaccine administration.

Alternatively, the vaccine composition may comprise one or more recombinantly-produced human CMV subunit proteins, preferably a fragment of a gB subunit. The *in vitro* produced subunit proteins may be introduced into the patient in a vaccine composition as described above, preferably employing the oral, nasal or subcutaneous routes of administration. The presence of the subunit produced either *in vivo* or as part of an *in vitro* expressed subunit administered with a carrier, stimulates an immune response in the patient. Such an immune response is capable of providing protection against exposure to the whole human CMV microorganism. The dosage for all routes of administration of the *in vitro* vaccine containing one or more of the CMV subunit proteins is generally greater than 20 micrograms of

protein per kg of patient body weight, and preferably between 40 and 80 micrograms of protein per kilogram.

The utility of the recombinant adenoviruses of the present invention is demonstrated through the use of a novel mouse experimental model which characterizes cytotoxic T lymphocyte (CTL) responses to individual proteins of strictly human-restricted viruses. For example, the model as used herein is based on the use of two types of recombinant viruses, an adenovirus and a canarypox virus, both expressing a gene of the same HCMV protein. This model is useful in identifying immunodominant HCMV proteins and immunodominant epitopes of individual proteins to incorporate into an appropriate immunizing vector, analysis of proteins of various HCMV strains, immunization protocols and the longevity of cell-mediated immunity to individual proteins or epitopes; and investigation of the optimal vector for effective introduction of a certain antigen or epitope to the host immune system.

According to this model, mice are immunized with one recombinant of the invention, and CTL activity is tested in target cells infected with the other recombinant. Specifically, Examples 4-6 below provide a murine model of the cytotoxic T lymphocyte (CTL) response to the amino acid 1-303 fragment of the glycoprotein B (gB) gene [SEQ ID NO:2] of human cytomegalovirus (HCMV) based on the use of gB-expressing adenovirus (Ad-gB) and several poxvirus recombinants. Using this model, it has been demonstrated that the human CMV subunit gB (HCMV-gB) amino acid 1-303 fragment can elicit a major histocompatibility complex (MHC) class I-restricted HCMV-gB-specific CTL response in mice.

The following examples illustrate the construction of a non-defective adenovirus strain capable of expressing the HCMV major envelope glycoprotein gB₁₋₃₀₃

fragment and the efficacy of these compositions as an HCMV vaccine. These examples are illustrative only and do not limit the scope of the present invention.

5 Example 1 - Construction of a Non-defective Adenovirus - gB (Ad-gB) Recombinant

 The gB gene was cloned from the Towne strain of HCMV [Wistar Institute] as follows. The gB gene was first mapped to the 20.5 kb Hind III D fragment of HCMV
10 using oligonucleotides that corresponded to the 5' and 3' termini of the published AD-169 gB sequence [See, Cranage et al (1986), cited above]. The Hind III fragment was cut with XbaI to generate a 9.8 kb fragment. This fragment was then cut with XmaIII to generate a 3.1 kb
15 fragment. The 3.1 kb XmaIII fragment which contained the gB gene, had XbaI linkers attached to its 5' and 3' termini.

 An adenovirus type 5 plasmid, pAd5 Bam-B, which contains the 59.5 - 100 mu region of the Ad5 adenovirus
20 genome cloned into the BamHI site of pBR322 [See, R. L. Berkner et al, Nucl. Acids Res., 11:6003-6020 (1983) and M. E. Morin et al, cited above] was digested with XbaI to remove the 78.5 mu - 84.7 mu sequences of the Ad5 genome. The 78.5 to 84.7 mu deletion removes most of the coding
25 region of the E3 transcription unit of Ad5 but leaves the E3 promoter intact. The XbaI-linked 3.1 kb fragment of CMV containing the gB gene was inserted into this XbaI site of pAd5 Bam-B. Fig. 1A provides a diagrammatic illustration of the above description.

30 To generate recombinant virus, the 0-76 mu fragment of wild type Ad5 virus was isolated by digesting the viral DNA with EcoRI [See, U. Petterson et al, J. Mol. Biol., 73:125-130 (1973)]. This fragment was co-transfected with the 59.5 to 100 mu BamHI fragment of
35 pAd5 Bam-B containing the gB gene as described above into

human embryonic kidney 293 cells, available from the American Type Culture Collection. The Ad-gB recombinant was generated by overlap recombination between the viral sequences as illustrated in Fig. 1B.

5 The gB recombinant virus was plaque purified on human lung carcinoma A549 cells [ATCC CCL185] using standard procedures. Viruses containing both orientations of the gB gene, as determined by Southern blotting, were isolated.

10 The recombinant containing the gB gene in the same 5' to 3' direction as the adenovirus E3 promoter of the adenovirus type 5 strain is under the transcriptional control of the E3 promoter. The plaque purified recombinant virus retains the cloning XbaI sites. The
15 above-described cloned gB gene is devoid of its natural promoter according to the DNA sequence of gB identified in Spaete et al, (1987), cited above.

Example 2 - Production of the Full-Length gB Subunit

20 The adenovirus gB plasmid construct and the Ad5 mu 0-76 DNA of Example 1 were cotransfected into 293 cells, human cells transformed by adenovirus 5 early genes [See, Graham et al, J. Gen. Virol., 36:59-72 (1977); and ATCC CRL1573] employing conventional procedures.
25 This transfection generated a functional recombinant virus by homologous overlap recombination as shown in Fig. 1B.

 Southern blot analysis confirmed the presence of an adenovirus, type 5, containing the HCMV gB subunit
30 (referred to as either Ad-5/gB or Ad-gB) recombinant virus which was subsequently purified by plaque purification using standard procedures.

 The recombinant virus AD-5/gB, expresses gB subunit protein as determined by conventional assays,
35 i.e., immunofluorescence on fixed cells and by Western

blot using monospecific guinea pig antiserum and monoclonal antibodies to gB protein [See, e.g., T. Maniatis et al, cited above]. The Ad-5/gB recombinant, also referred to as Ad-gB, is also described in applicant's publication [Marshall et al., J. Infect. Dis., 162:1177-1181 (1990)] published after the filing date of the original parent application from which this application claims priority.

10. Example 3 - Construction of the gB gene fragments

Ad-gB₁₋₃₀₃ and Ad-gB₁₋₁₅₅ recombinant viruses were constructed by overlap recombination as described for Ad-gB in Example 2 above. Briefly, in order to clone the subfragments of the gB gene, five oligonucleotide primers for polymerase chain reactions (PCR) were synthesized. The primers were designed to anneal with various portions of the gB DNA sequence and promote amplification of the gene. In addition, all of the oligonucleotide primers were engineered to contain an Xba I site so that the PCR product could be digested with this enzyme in order to facilitate cloning into the pAd-5 vector.

5' gB primer : SEQ ID NO:3:

4889: 5'-ACACGCAAGAGA TCTAGA CGCGCCTCAT

3' primer at amino acid 700 of gB protein: SEQ ID NO:4:

25 5'-TCGTCCAGAC TCTAGA GGTAGGGC

3' primer at aa 465: SEQ ID NO:5:

5'-CGACTCCAT TCTAGA TTAATGAGTTGCATT

3' primer at aa 303: SEQ ID NO:6:

5'-CAAAGTCGGAG TCTAGAG TCTAGTTCGGAAA

30 3' primer at aa 155: SEQ ID NO:7:

5'-CAGATAAGTGG TCTAGA TCTAAGCGTAGCTACG

The above oligonucleotides correspond to the following nucleotide positions of the HCMV gB gene (Towne strain) as reported by Spaete et al, Virology, 167:207-225

35 (1988). SEQ ID NO:3 corresponds to nucleotide positions

895 to 922 in the sense orientation; SEQ ID NO:4 to nucleotide positions 3090 to 3067 anti-sense; SEQ ID NO:5 to nucleotide positions 2375 to 2350 anti-sense; SEQ ID NO:6 to nucleotide positions 1877 to 1847 anti-sense; and
5 SEQ ID NO:7 to nucleotide positions 1432 to 1400 anti-sense. These immediately preceding nucleotide numbers are not identical to those of SEQ ID NO: 1 because the Spaete et al sequence, to which these numbers correspond, contains additional 5' non-coding sequence while SEQ ID
10 NO: 1 reports only the DNA sequence corresponding to the coding region of the gB protein [SEQ ID NO: 2].

The specific segments or fragments of the gB gene were amplified using the Perkin-Elmer Amplitaq™ kit by mixing 400 ng of the 5' gB primer with each of the 3'
15 primers separately (400 ng of each) and 0.1 µg of purified HCMV genomic DNA or 0.1 µg of previously cloned intact gB gene (see Example 2). The final reaction mixture was 100 µL and the thermocycling conditions were 94°C, 1 minute; 52°C, 1 minute; 72°C, 1 minute, repeated
20 for a total of 35 cycles. Amplified DNA was purified by cutting the proper DNA fragment out of a 1.2% agarose gel, digested with *Xba*I, repurified by cutting the digested fragments out of a 1.2% agarose gel and then ligated into the *Xba*I site of the cloning vector pAd-5.
25 Positive recombinants were verified by DNA sequence analysis and sequence analysis confirmed the orientation of the clones.

Example 4 - CTL Assays

30 A. Recombinant Viruses Used

The following recombinant viruses were used in the CTL assays of Examples 5-6 below to demonstrate the immunogenicity and vaccine utility of the recombinant adenoviruses of the present invention.

Wild-type human adenovirus type 5 (WT-Ad) and the Ad-gB recombinant were propagated in human lung carcinoma A549 cells [ATCC CCL185], as described in Example 1.

5 An E3-deleted adenovirus type 5 mutant lacking the XbaI D fragment of adenovirus DNA (Ad5ΔE3) was constructed by overlap recombination, using plasmid pAd-5 mu 59.5-100, which was deleted in E3 sequences (mu 78.5-84) using the techniques described in Example 1, and
10 pAd-5 mu 0-75.9 [G. S. Marshall et al, J. Infect. Dis., 162:1177-1181 (1990), hereby incorporated by reference].

 A vaccinia virus recombinant containing the gB subunits (VacC-gB) described previously in Gonczol et al, Vaccine, 9:631-637 (1991) and the parental Copenhagen
15 strain of vaccinia, VC-2 (also known as wild-type vaccinia (WT-Vac)) were grown in Vero cells [E. Gonczol et al, Vaccine, 8:130-136 (1990); J. Tartaglia et al, Crit. Rev. Immunol., 10:13-30 (1990)].

 The vaccinia WR strain [obtained from Dr. Enzo
20 Paoletti, Virogenetics Corp, Troy, NY] was used to develop a recombinant expressing HCMV-gB ((VacW)-gB). This recombinant was derived using a strategy similar to that described for the VacC-gB recombinant (Gonczol et al., cited above).

25 A canarypox recombinant [ALVAC-CMV (vCP139) which is subsequently referred to as Cp-gB] expressing the HCMV-gB gene was constructed using a strategy similar to that described for a canarypox-rabies recombinant in Taylor et al., Vaccine, 9:190-193 (1991) [also obtained
30 from Dr. Enzo Paoletti]. Briefly, the gene encoding the HCMV (Towne strain) glycoprotein B was inserted into a canarypox donor plasmid consisting of a polylinker flanked by genomic sequence from which a nonessential gene was specifically deleted (at a unique EcoRI site
35 within a 3.3 kbp PvuII subgenomic fragment of canarypox

DNA). Expression of the gB protein gene was placed under the transcriptional control of an early/late vaccinia virus promoter (H6) previously described [Percus et al., J. Virol., 63:3829-3835 (1989)]. Cp-gB was derived and plaque-purified by standard methods [Panicali and Paoletti, Proc. Natl. Acad. Sci. USA, 79:4927-4931 (1982)]. The Cp-gB recombinant and parental canarypox virus (WT-Cp) were propagated on primary chick embryo fibroblasts.

10 B. Expression of the gB protein in Cp-gB recombinant virus

Chicken embryo fibroblast (CEF) cells [ATCC CRL 1590] infected with either Cp-gB or with the parental wild-type canarypox (WT-Cp) virus preparations were analyzed by Western blot assay using the 4A guinea-pig serum directed against the gB protein. Western blot assays and the 4A guinea-pig serum, used as gB-specific antibody, were described previously in Gonczol et al., J. Virol., 58:661-664 (1986). Uninfected and HCMV-infected MRC-5 cell [ATCC CCL 171] lysates were included as controls.

A diffuse band at the 140 kDa position and a double band of 55 and 58 kDa were detected in both Cp-gB-infected CEF cells and in HCMV-infected MRC-5 cells. The presence of these gB-specific proteins presumably representing the glycosylated 140 kDa precursor and the differentially glycosylated cleavage products (55 and 58 kDa) indicates that the Cp-gB recombinant expresses the inserted gB gene. The slight difference between the mobility of 55 and 58 kDa cleavage products of control and recombinant gB may reflect different glycosylation patterns.

C. Murine Model and CTL Assay

For immunization of mice, Ad-gB and WT-Ad were purified by CsCl gradient centrifugation. VacC-gB,

VacW-gB and WT-Vac were purified by sucrose gradient centrifugation, and Cp-gB and WT-Cp were concentrated on sucrose cushion.

Six- to 8-week-old female BALB/c and CBA mice (from Harlan Sprague-Dawley and Jackson) and 12-week-old male BALB/k mice (from The Wistar Institute Animal Facility) were immunized intraperitoneally (i.p.) with the recombinant viruses described above at $1-5 \times 10^8$ pfu unless otherwise stated.

One to 12 weeks later, spleens were aseptically removed and cell suspensions were prepared by gently pressing the spleens through a stainless steel mesh. Cells were suspended at 2.5×10^6 viable cells/ml in RPMI 1640 medium containing 5% FBS (Gibco), 2×10^{-5} M 2-mercaptoethanol, 14 mM HEPES buffer, glutamine and 50 μ g/ml gentamicin. Spleen cell cultures were restimulated *in vitro* with Ad-gB (multiplicity of infection (m.o.i.) = 10) or VacC-gB (m.o.i. = 0.5) infected autologous spleen cells for 5 days in 24-well plates. Cytolytic activity of nonadherent spleen cells was tested in a chromium release assay which was performed as follows.

1. T-cell subset depletion

For *in vitro* depletion of CD4 or CD8 cells, 3×10^6 spleen cells were incubated with anti-mouse CD4 monoclonal antibody (MAB) [Pharmingen; Cat.3:01061 D; 20 μ g/ 3×10^6 cells] or CD8 MAB [Accurate; Cat. #:CL-8921; diluted 1:4] for 60 minutes at 4°C, and further incubated in the presence of rabbit complement [Accurate; Low-tox M; diluted 1:10] for 30 minutes at 37°C. The cells were washed twice and used as effector cells in a ^{51}Cr -release test.

2. Chromium release assay

P815 (H-2^d) [ATCC TIB 64], mouse MC57 (H-2^b) cells [also termed MC-57G, D.P. Aden et al, Immunogenetics, 3:209-221 (1976)] and mouse NCTC clone

929 (H-2^k) cells [ATCC CCL 1] were used as target cells. The HCMV neutralization titer of mouse sera was determined on MRC-5 cells [ATCC CCL 171] by the microneutralization method as described in Gonczol et al., J. Virol. Methods, 14:37-41 (1986).

The target cells were infected with Ad-gB or Ad-5ΔE3 (multiplicity of infection (m.o.i.) = 40-80, 40 hours) or with Vac-gB or WT-Vac (m.o.i. = 5-10, 4 hours). Target cells were washed in the modified RPMI 1640 medium described above and 2 x 10⁶ cells were labeled with 100 μCi of [⁵¹Cr]NaCrO₄ [Amersham, specific activity 250-500 mCi/mg] for 1 hour. The labeled target cells were washed 3 times in phosphate-buffered saline (PBS) and then mixed with the effector cells at various effector:target ratios in triplicate using 96-well U-bottomed microtiter plates and incubated for 4 hours.

Percentage specific ⁵¹Cr release was calculated as: [(cpm experimental release - cpm spontaneous release) / (cpm maximal release - cpm spontaneous release)] x 100. Standard deviation of the mean of triplicate cultures was less than 10%, and spontaneous release was always less than 25%.

This CTL assay is a system in which two types of viral expression vectors, poxvirus and adenovirus, carrying the same fragment of the HCMV-gB gene, are alternately used for immunization of animal or for infection of target cells to show that HCMV-gB fragment is an inducer of CTL in mice. Using this model system, the relative immunogenicity of the gB fragment expressed by different recombinant viruses has been evaluated.

Example 5 - CTL Responses to Adenovirus Containing gB Fragments

Ad-gB₁₋₃₀₃ and Ad-gB₁₋₁₅₅ recombinant viruses were constructed as described in Example 3 above.

In CTL experiments performed as described in Example 4, CBA mice were immunized i.p. with 10^8 pfu of the Ad-gB, Ad-gB₁₋₃₀₃ or Ad-gB₁₋₁₅₅. Two weeks later spleen cells were restimulated in vitro with Ad-gB infected autologous spleen cells and tested for ability to lyse Wt-Ad, Vac-gB or Wt-Vac infected L929 (MHC-class I matched) cells.

All recombinants showed an Ad virus-specific CTL response, but only Ad-gB (containing the complete gB coding sequence) and Ad-gB₁₋₃₀₃ exerted gB-specific CTL, indicating the presence of a CTL-epitope on the N-terminal part of the gB protein between amino acid 155 and 303.

Example 6 - Protection Studies with Adenovirus Containing gB Fragments

Using the murine model described in Example 4, CBA mice were immunized with 1×10^8 pfu of Wt-Ad, Ad5 Δ 3 (an E3 deleted mutant virus, the parental strain of the recombinant viruses), Ad-gB, Ad-gB₁₋₃₀₃ or Ad-gB₁₋₁₅₅. Five to ten days later the immunized mice were challenged i.c. with VacWR-gB (a neurovirulent vaccinia strain expressing the HCMV-gB protein). Control mice, immunized with the Wt-Ad or Ad5 Δ 3 virus died within 4-7 days after the challenge.

Ad-gB and Ad-gB₁₋₃₀₃-immunized mice survived (92% and 95% survival, respectively), while all of the Ad-gB₁₋₁₅₅-immunized mice died, indicating a protection epitope on the N-terminal part of the gB protein between amino acid 155 and 303.

Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. For example, use of other appropriate non-defective adenovirus strains for construction of

analogous expression systems to express the HCMV gB fragment may be constructed according to the disclosure of the present invention.

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Additionally, the other subunits of HCMV major glycoprotein complexes, e.g., gCII or gCIII, or immediate-early antigens, may be expressed in a non-defective adenovirus recombinant in the same manner as described above for subunit gB fragment. Such
10 modifications and alterations to the compositions and processes of the present invention are believed to be encompassed in the scope of the claims appended hereto.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Wistar Institute of Anatomy, Biology
Government of USA Dept.
Health and Human Services
- (ii) TITLE OF INVENTION: Recombinant Cytomegalovirus
Vaccine
- (iii) NUMBER OF SEQUENCES: 7
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Howson and Howson
 - (B) STREET: Spring House Corporate Center, PO Box 457
 - (C) CITY: Spring House
 - (D) STATE: Pennsylvania
 - (E) COUNTRY: USA
 - (F) ZIP: 19477
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/048,978
 - (B) FILING DATE: 16-APR-1993
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Bak, Mary E.
 - (B) REGISTRATION NUMBER: 31,215
 - (C) REFERENCE/DOCKET NUMBER: WST6CPCT
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 215-540-9200
 - (B) TELEFAX: 215-540-5818

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2724 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..2721

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

| | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| ATG | GAA | TCC | AGG | ATC | TGG | TGC | CTG | GTA | GTC | TGC | GTT | AAC | TTG | 42 |
| Met | Glu | Ser | Arg | Ile | Trp | Cys | Leu | Val | Val | Cys | Val | Asn | Leu | |
| 1 | | | | 5 | | | | | 10 | | | | | |
| TGT | ATC | GTC | TGT | CTG | GGT | GCT | GCG | GTT | TCC | TCA | TCT | TCT | ACT | 84 |
| Cys | Ile | Val | Cys | Leu | Gly | Ala | Ala | Val | Ser | Ser | Ser | Ser | Thr | |
| 15 | | | | 20 | | | | | 25 | | | | | |
| CGT | GGA | ACT | TCT | GCT | ACT | CAC | AGT | CAC | CAT | TCC | TCT | CAT | ACG | 126 |
| Arg | Gly | Thr | Ser | Ala | Thr | His | Ser | His | His | Ser | Ser | His | Thr | |
| | 30 | | | | | 35 | | | | | 40 | | | |
| ACG | TCT | GCT | GCT | CAT | TCT | CGA | TCC | GGT | TCA | GTC | TCT | CAA | CGC | 168 |
| Thr | Ser | Ala | Ala | His | Ser | Arg | Ser | Gly | Ser | Val | Ser | Gln | Arg | |
| | | 45 | | | | 50 | | | | | | 55 | | |
| GTA | ACT | TCT | TCC | CAA | ACG | GTC | AGC | CAT | GGT | GTT | AAC | GAG | ACC | 210 |
| Val | Thr | Ser | Ser | Gln | Thr | Val | Ser | His | Gly | Val | Asn | Glu | Thr | |
| | | | 60 | | | | | 65 | | | | | 70 | |
| ATC | TAC | AAC | ACT | ACC | CTC | AAG | TAC | GGA | GAT | GTG | GTG | GGG | GTC | 252 |
| Ile | Tyr | Asn | Thr | Thr | Leu | Lys | Tyr | Gly | Asp | Val | Val | Gly | Val | |
| | | | | 75 | | | | 80 | | | | | | |
| AAC | ACC | ACC | AAG | TAC | CCC | TAT | CGC | GTG | TGT | TCT | ATG | GCA | CAG | 294 |
| Asn | Thr | Thr | Lys | Tyr | Pro | Tyr | Arg | Val | Cys | Ser | Met | Ala | Gln | |
| | 85 | | | | 90 | | | | | 95 | | | | |
| GGT | ACG | GAT | CTT | ATT | CGC | TTT | GAA | CGT | AAT | ATC | GTC | TGC | ACC | 336 |
| Gly | Thr | Asp | Leu | Ile | Arg | Phe | Glu | Arg | Asn | Ile | Val | Cys | Thr | |
| | 100 | | | | 105 | | | | | | 110 | | | |
| TCG | ATG | AAG | CCC | ATC | AAT | GAA | GAC | CTG | GAC | GAG | GGC | ATC | ATG | 378 |
| Ser | Met | Lys | Pro | Ile | Asn | Glu | Asp | Leu | Asp | Glu | Gly | Ile | Met | |
| | | 115 | | | | 120 | | | | | | 125 | | |

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|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| GTG | GTC | TAC | AAA | CGC | AAC | ATC | GTC | GCG | CAC | ACC | TTT | AAG | GTA | 420 |
| Val | Val | Tyr | Lys | Arg | Asn | Ile | Val | Ala | His | Thr | Phe | Lys | Val | |
| | | | 130 | | | | | 135 | | | | | 140 | |
| CGA | GTC | TAC | CAG | AAG | GTT | TTG | ACG | TTT | CGT | CGT | AGC | TAC | GCT | 462 |
| Arg | Val | Tyr | Gln | Lys | Val | Leu | Thr | Phe | Arg | Arg | Ser | Tyr | Ala | |
| | | | 145 | | | | | 150 | | | | | | |
| TAC | ATC | CAC | ACC | ACT | TAT | CTG | CTG | GGC | AGC | AAC | ACG | GAA | TAC | 504 |
| Tyr | Ile | His | Thr | Thr | Tyr | Leu | Leu | Gly | Ser | Asn | Thr | Glu | Tyr | |
| 155 | | | | | 160 | | | | | 165 | | | | |
| GTG | GCG | CCT | CCT | ATG | TGG | GAG | ATT | CAT | CAT | ATC | AAC | AGT | CAC | 546 |
| Val | Ala | Pro | Pro | Met | Trp | Glu | Ile | His | His | Ile | Asn | Ser | His | |
| | 170 | | | | | 175 | | | | | 180 | | | |
| AGT | CAG | TGC | TAC | AGT | TCC | TAC | AGC | CGC | GTT | ATA | GCA | GGC | ACG | 588 |
| Ser | Gln | Cys | Tyr | Ser | Ser | Tyr | Ser | Arg | Val | Ile | Ala | Gly | Thr | |
| | | 185 | | | | | 190 | | | | | 195 | | |
| GTT | TTC | GTG | GCT | TAT | CAT | AGG | GAC | AGC | TAT | GAA | AAC | AAA | ACC | 630 |
| Val | Phe | Val | Ala | Tyr | His | Arg | Asp | Ser | Tyr | Glu | Asn | Lys | Thr | |
| | | | 200 | | | | | 205 | | | | | 210 | |
| ATG | CAA | TTA | ATG | CCC | GAC | GAT | TAT | TCC | AAC | ACC | CAC | AGT | ACC | 672 |
| Met | Gln | Leu | Met | Pro | Asp | Asp | Tyr | Ser | Asn | Thr | His | Ser | Thr | |
| | | | | 215 | | | | | 220 | | | | | |
| CGT | TAC | GTG | ACG | GTC | AAG | GAT | CAA | TGG | CAC | AGC | CGC | GGC | AGC | 714 |
| Arg | Tyr | Val | Thr | Val | Lys | Asp | Gln | Trp | His | Ser | Arg | Gly | Ser | |
| 225 | | | | | 230 | | | | | 235 | | | | |
| ACC | TGG | CTC | TAT | CGT | GAG | ACC | TGT | AAT | CTG | AAT | TGT | ATG | GTG | 756 |
| Thr | Trp | Leu | Tyr | Arg | Glu | Thr | Cys | Asn | Leu | Asn | Cys | Met | Val | |
| | 240 | | | | 245 | | | | | 250 | | | | |
| ACC | ATC | ACT | ACT | GCG | CGC | TCC | AAG | TAT | CCC | TAT | CAT | TTT | TTC | 798 |
| Thr | Ile | Thr | Thr | Ala | Arg | Ser | Lys | Tyr | Pro | Tyr | His | Phe | Phe | |
| | | 255 | | | | | 260 | | | | | 265 | | |
| GCA | ACT | TCC | ACG | GGT | GAT | GTG | GTT | GAC | ATT | TCT | CCT | TTC | TAC | 840 |
| Ala | Thr | Ser | Thr | Gly | Asp | Val | Val | Asp | Ile | Ser | Pro | Phe | Tyr | |
| | | | 270 | | | | | 275 | | | | | 280 | |
| AAC | GGA | ACT | AAT | CGC | AAT | GCC | AGC | TAT | TTT | GGA | GAA | AAC | GCC | 882 |
| Asn | Gly | Thr | Asn | Arg | Asn | Ala | Ser | Tyr | Phe | Gly | Glu | Asn | Ala | |
| | | | | 285 | | | | | 290 | | | | | |
| GAC | AAG | TTT | TTC | ATT | TTT | CCG | AAC | TAC | ACT | ATC | GTC | TCC | GAC | 924 |
| Asp | Lys | Phe | Phe | Ile | Phe | Pro | Asn | Tyr | Thr | Ile | Val | Ser | Asp | |
| 295 | | | | | 300 | | | | | 305 | | | | |

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|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| TTT | GGA | AGA | CCG | AAT | TCT | GCG | TTA | GAG | ACC | CAC | AGG | TTG | GTG | 966 |
| Phe | Gly | Arg | Pro | Asn | Ser | Ala | Leu | Glu | Thr | His | Arg | Leu | Val | |
| 310 | | | | | | 315 | | | | | 320 | | | |
| GCT | TTT | CTT | GAA | CGT | GCG | GAC | TCA | GTG | ATC | TCC | TGG | GAT | ATA | 1008 |
| Ala | Phe | Leu | Glu | Arg | Ala | Asp | Ser | Val | Ile | Ser | Trp | Asp | Ile | |
| 325 | | | | | | 330 | | | | | 335 | | | |
| CAG | GAC | GAG | AAG | AAT | GTT | ACT | TGT | CAA | CTC | ACT | TTC | TGG | GAA | 1050 |
| Gln | Asp | Glu | Lys | Asn | Val | Thr | Cys | Gln | Leu | Thr | Phe | Trp | Glu | |
| 340 | | | | | | | 345 | | | | | | 350 | |
| GCC | TCG | GAA | CGC | ACC | ATT | CGT | TCC | GAA | GCC | GAG | GAC | TCG | TAT | 1092 |
| Ala | Ser | Glu | Arg | Thr | Ile | Arg | Ser | Glu | Ala | Glu | Asp | Ser | Tyr | |
| | | | | 355 | | | | 360 | | | | | | |
| CAC | TTT | TCT | TCT | GCC | AAA | ATG | ACC | GCC | ACT | TTC | TTA | TCT | AAG | 1134 |
| His | Phe | Ser | Ser | Ala | Lys | Met | Thr | Ala | Thr | Phe | Leu | Ser | Lys | |
| 365 | | | | | 370 | | | | | 375 | | | | |
| AAG | CAA | GAG | GTG | AAC | ATG | TCC | GAC | TCT | GCG | CTG | GAC | TGT | GTA | 1176 |
| Lys | Gln | Glu | Val | Asn | Met | Ser | Asp | Ser | Ala | Leu | Asp | Cys | Val | |
| 380 | | | | | | 385 | | | | | 390 | | | |
| CGT | GAT | GAG | GCC | ATA | AAT | AAG | TTA | CAG | CAG | ATT | TTC | AAT | ACT | 1218 |
| Arg | Asp | Glu | Ala | Ile | Asn | Lys | Leu | Gln | Gln | Ile | Phe | Asn | Thr | |
| | 395 | | | | | | 400 | | | | | 405 | | |
| TCA | TAC | AAT | CAA | ACA | TAT | GAA | AAA | TAT | GGA | AAC | GTG | TCC | GTC | 1260 |
| Ser | Tyr | Asn | Gln | Thr | Tyr | Glu | Lys | Tyr | Gly | Asn | Val | Ser | Val | |
| | | | 410 | | | | | 415 | | | | | 420 | |
| TTT | GAA | ACC | ACT | GGT | GGT | TTG | GTG | GTG | TTC | TGG | CAA | GGT | ATC | 1302 |
| Phe | Glu | Thr | Thr | Gly | Gly | Leu | Val | Val | Phe | Trp | Gln | Gly | Ile | |
| | | | | 425 | | | | 430 | | | | | | |
| AAG | CAA | AAA | TCT | CTG | GTG | GAA | CTC | GAA | CGT | TTG | GCC | AAC | CGC | 1344 |
| Lys | Gln | Lys | Ser | Leu | Val | Glu | Leu | Glu | Arg | Leu | Ala | Asn | Arg | |
| 435 | | | | | 440 | | | | | 445 | | | | |
| TCC | AGT | CTG | AAT | CTT | ACT | CAT | AAT | AGA | ACC | AAA | AGA | AGT | ACA | 1386 |
| Ser | Ser | Leu | Asn | Leu | Thr | His | Asn | Arg | Thr | Lys | Arg | Ser | Thr | |
| | 450 | | | | | 455 | | | | | 460 | | | |
| GAT | GGC | AAC | AAT | GCA | ACT | CAT | TTA | TCC | AAC | ATG | GAG | TCG | GTG | 1428 |
| Asp | Gly | Asn | Asn | Ala | Thr | His | Leu | Ser | Asn | Met | Glu | Ser | Val | |
| | | 465 | | | | | 470 | | | | | 475 | | |
| CAC | AAT | CTG | GTC | TAC | GCC | CAG | CTG | CAG | TTC | ACC | TAT | GAC | ACG | 1470 |
| His | Asn | Leu | Val | Tyr | Ala | Gln | Leu | Gln | Phe | Thr | Tyr | Asp | Thr | |
| | | | 480 | | | | | 485 | | | | | 490 | |

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|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| TTG | CGC | GGT | TAC | ATC | AAC | CGG | GCG | CTG | GCG | CAA | ATC | GCA | GAA | 1512 |
| Leu | Arg | Gly | Tyr | Ile | Asn | Arg | Ala | Leu | Ala | Gln | Ile | Ala | Glu | |
| | | | | 495 | | | | | 500 | | | | | |
| GCC | TGG | TGT | GTG | GAT | CAA | CGG | CGC | ACC | CTA | GAG | GTC | TTC | AAG | 1554 |
| Ala | Trp | Cys | Val | Asp | Gln | Arg | Arg | Thr | Leu | Glu | Val | Phe | Lys | |
| 505 | | | | | 510 | | | | | 515 | | | | |
| GAA | CTT | AGC | AAG | ATC | AAC | CCG | TCA | GCT | ATT | CTC | TCG | GCC | ATC | 1596 |
| Glu | Leu | Ser | Lys | Ile | Asn | Pro | Ser | Ala | Ile | Leu | Ser | Ala | Ile | |
| | 520 | | | | | 525 | | | | | 530 | | | |
| TAC | AAC | AAA | CCG | ATT | GCC | GCG | CGT | TTC | ATG | GGT | GAT | GTC | CTG | 1638 |
| Tyr | Asn | Lys | Pro | Ile | Ala | Ala | Arg | Phe | Met | Gly | Asp | Val | Leu | |
| | | 535 | | | | | 540 | | | | | 545 | | |
| GGT | CTG | GCC | AGC | TGC | GTG | ACC | ATT | AAC | CAA | ACC | AGC | GTC | AAG | 1680 |
| Gly | Leu | Ala | Ser | Cys | Val | Thr | Ile | Asn | Gln | Thr | Ser | Val | Lys | |
| | | | 550 | | | | | 555 | | | | | 560 | |
| GTG | CTG | CGT | GAT | ATG | AAT | GTG | AAG | GAA | TCG | CCA | GGA | CGC | TGC | 1722 |
| Val | Leu | Arg | Asp | Met | Asn | Val | Lys | Glu | Ser | Pro | Gly | Arg | Cys | |
| | | | | 565 | | | | | 570 | | | | | |
| TAC | TCA | CGA | CCA | GTG | GTC | ATC | TTT | AAT | TTC | GCC | AAC | AGC | TCG | 1764 |
| Tyr | Ser | Arg | Pro | Val | Val | Ile | Phe | Asn | Phe | Ala | Asn | Ser | Ser | |
| 575 | | | | | 580 | | | | | 585 | | | | |
| TAC | GTG | CAG | TAC | GGT | CAA | CTG | GGC | GAG | GAT | AAC | GAA | ATC | CTG | 1806 |
| Tyr | Val | Gln | Tyr | Gly | Gln | Leu | Gly | Glu | Asp | Asn | Glu | Ile | Leu | |
| | 590 | | | | | 595 | | | | | 600 | | | |
| TTG | GGC | AAC | CAC | CGC | ACT | GAG | GAA | TGT | CAG | CTT | CCC | AGC | CTC | 1848 |
| Leu | Gly | Asn | His | Arg | Thr | Glu | Glu | Cys | Gln | Leu | Pro | Ser | Leu | |
| | | 605 | | | | | 610 | | | | | 615 | | |
| AAG | ATC | TTC | ATC | GCC | GGC | AAC | TCG | GCC | TAC | GAG | TAC | GTG | GAC | 1890 |
| Lys | Ile | Phe | Ile | Ala | Gly | Asn | Ser | Ala | Tyr | Glu | Tyr | Val | Asp | |
| | | | 620 | | | | | 625 | | | | | 630 | |
| TAC | CTC | TTC | AAA | CGC | ATG | ATT | GAC | CTC | AGC | AGC | ATC | TCC | ACC | 1932 |
| Tyr | Leu | Phe | Lys | Arg | Met | Ile | Asp | Leu | Ser | Ser | Ile | Ser | Thr | |
| | | | | 635 | | | | | 640 | | | | | |
| GTC | GAC | AGC | ATG | ATC | GCC | CTA | GAC | ATC | GAC | CCG | CTG | GAA | AAC | 1974 |
| Val | Asp | Ser | Met | Ile | Ala | Leu | Asp | Ile | Asp | Pro | Leu | Glu | Asn | |
| 645 | | | | | 650 | | | | | 655 | | | | |
| ACC | GAC | TTC | AGG | GTA | CTG | GAA | CTT | TAC | TCG | CAG | AAA | GAA | TTG | 2016 |
| Thr | Asp | Phe | Arg | Val | Leu | Glu | Leu | Tyr | Ser | Gln | Lys | Glu | Leu | |
| | 660 | | | | | 665 | | | | | 670 | | | |

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|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| CGT | TCC | AGC | AAC | GTT | TTT | GAT | CTC | GAG | GAG | ATC | ATG | CGC | GAG | 2058 |
| Arg | Ser | Ser | Asn | Val | Phe | Asp | Leu | Glu | Glu | Ile | Met | Arg | Glu | |
| | | 675 | | | | | 680 | | | | | 685 | | |
| TTC | AAT | TCG | TAT | AAG | CAG | CGG | GTA | AAG | TAC | GTG | GAG | GAC | AAG | 2100 |
| Phe | Asn | Ser | Tyr | Lys | Gln | Arg | Val | Lys | Tyr | Val | Glu | Asp | Lys | |
| | | | 690 | | | | | 695 | | | | | 700 | |
| GTA | GTC | GAC | CCG | CTG | CCG | CCC | TAC | CTC | AAG | GGT | CTG | GAC | GAC | 2142 |
| Val | Val | Asp | Pro | Leu | Pro | Pro | Tyr | Leu | Lys | Gly | Leu | Asp | Asp | |
| | | | 705 | | | | | | 710 | | | | | |
| CTC | ATG | AGC | GGC | CTG | GGC | GCC | GCG | GGA | AAG | GCC | GTT | GGC | GTA | 2184 |
| Leu | Met | Ser | Gly | Leu | Gly | Ala | Ala | Gly | Lys | Ala | Val | Gly | Val | |
| 715 | | | | | 720 | | | | | 725 | | | | |
| GCC | ATT | GGG | GCC | GTG | GGT | GGC | GCG | GTG | GCC | TCC | GTG | GTC | GAA | 2226 |
| Ala | Ile | Gly | Ala | Val | Gly | Gly | Ala | Val | Ala | Ser | Val | Val | Glu | |
| 730 | | | | | | 735 | | | | | 740 | | | |
| GGC | GTT | GCC | ACC | TTC | CTC | AAA | AAC | CCC | TTC | GGA | GCC | TTC | ACC | 2268 |
| Gly | Val | Ala | Thr | Phe | Leu | Lys | Asn | Pro | Phe | Gly | Ala | Phe | Thr | |
| | | 745 | | | | | 750 | | | | | 755 | | |
| ATC | ATC | CTC | GTG | GCC | ATA | GCC | GTC | GTC | ATT | ATC | ATT | TAT | TTG | 2310 |
| Ile | Ile | Leu | Val | Ala | Ile | Ala | Val | Val | Ile | Ile | Ile | Tyr | Leu | |
| | | | 760 | | | | | 765 | | | | | 770 | |
| ATC | TAT | ACT | CGA | CAG | CGG | CGT | CTC | TGC | ATG | CAG | CCG | CTG | CAG | 2352 |
| Ile | Tyr | Thr | Arg | Gln | Arg | Arg | Leu | Cys | Met | Gln | Pro | Leu | Gln | |
| | | | 775 | | | | | | 780 | | | | | |
| AAC | CTC | TTT | CCC | TAT | CTG | GTG | TCC | GCC | GAC | GGG | ACC | ACC | GTG | 2394 |
| Asn | Leu | Phe | Pro | Tyr | Leu | Val | Ser | Ala | Asp | Gly | Thr | Thr | Val | |
| 785 | | | | | 790 | | | | | 795 | | | | |
| ACG | TCG | GGC | AAC | ACC | AAA | GAC | ACG | TCG | TTA | CAG | GCT | CCG | CCT | 2436 |
| Thr | Ser | Gly | Asn | Thr | Lys | Asp | Thr | Ser | Leu | Gln | Ala | Pro | Pro | |
| | | 800 | | | | 805 | | | | | 810 | | | |
| TCC | TAC | GAG | GAA | AGT | GTT | TAT | AAT | TCT | GGT | CGC | AAA | GGA | CCG | 2478 |
| Ser | Tyr | Glu | Glu | Ser | Val | Tyr | Asn | Ser | Gly | Arg | Lys | Gly | Pro | |
| | | 815 | | | | | 820 | | | | | 825 | | |
| GGA | CCA | CCG | TCG | TCT | GAT | GCA | TCC | ACG | GCG | GCT | CCG | CCT | TAC | 2520 |
| Gly | Pro | Pro | Ser | Ser | Asp | Ala | Ser | Thr | Ala | Ala | Pro | Pro | Tyr | |
| | | | 830 | | | | | 835 | | | | | 840 | |
| ACC | AAC | GAG | CAG | GCT | TAC | CAG | ATG | CTT | CTG | GCC | CTG | GTC | CGT | 2562 |
| Thr | Asn | Glu | Gln | Ala | Tyr | Gln | Met | Leu | Leu | Ala | Leu | Val | Arg | |
| | | | | 845 | | | | | 850 | | | | | |

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| | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| CTG | GAC | GCA | GAG | CAG | CGA | GCG | CAG | CAG | AAC | GGT | ACA | GAT | TCT | 2604 |
| Leu | Asp | Ala | Glu | Gln | Arg | Ala | Gln | Gln | Asn | Gly | Thr | Asp | Ser | |
| 855 | | | | | 860 | | | | | 865 | | | | |
| | | | | | | | | | | | | | | |
| TTG | GAC | GGA | CAG | ACT | GGC | ACG | CAG | GAC | AAG | GGA | CAG | AAG | CCC | 2646 |
| Leu | Asp | Gly | Gln | Thr | Gly | Thr | Gln | Asp | Lys | Gly | Gln | Lys | Pro | |
| 870 | | | | | 875 | | | | | 880 | | | | |
| | | | | | | | | | | | | | | |
| AAC | CTG | CTA | GAC | CGA | CTG | CGA | CAC | CGC | AAA | AAC | GGC | TAC | CGA | 2688 |
| Asn | Leu | Leu | Asp | Arg | Leu | Arg | His | Arg | Lys | Asn | Gly | Tyr | Arg | |
| | | 885 | | | | | 890 | | | | | 895 | | |
| | | | | | | | | | | | | | | |
| CAC | TTG | AAA | GAC | TCC | GAC | GAA | GAA | GAG | AAC | GTC | TGA | | | 2724 |
| His | Leu | Lys | Asp | Ser | Asp | Glu | Glu | Glu | Asn | Val | | | | |
| | | | 900 | | | | | 905 | | | | | | |

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 907 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Glu | Ser | Arg | Ile | Trp | Cys | Leu | Val | Val | Cys | Val | Asn | Leu | Cys | Ile |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
| | | | | | | | | | | | | | | | |
| Val | Cys | Leu | Gly | Ala | Ala | Val | Ser | Ser | Ser | Ser | Thr | Arg | Gly | Thr | Ser |
| | | | 20 | | | | | 25 | | | | | 30 | | |
| | | | | | | | | | | | | | | | |
| Ala | Thr | His | Ser | His | His | Ser | Ser | His | Thr | Thr | Ser | Ala | Ala | His | Ser |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| | | | | | | | | | | | | | | | |
| Arg | Ser | Gly | Ser | Val | Ser | Gln | Arg | Val | Thr | Ser | Ser | Gln | Thr | Val | Ser |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| | | | | | | | | | | | | | | | |
| His | Gly | Val | Asn | Glu | Thr | Ile | Tyr | Asn | Thr | Thr | Leu | Lys | Tyr | Gly | Asp |
| 65 | | | | | 70 | | | | | 75 | | | | 80 | |
| | | | | | | | | | | | | | | | |
| Val | Val | Gly | Val | Asn | Thr | Thr | Lys | Tyr | Pro | Tyr | Arg | Val | Cys | Ser | Met |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| | | | | | | | | | | | | | | | |
| Ala | Gln | Gly | Thr | Asp | Leu | Ile | Arg | Phe | Glu | Arg | Asn | Ile | Val | Cys | Thr |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| | | | | | | | | | | | | | | | |
| Ser | Met | Lys | Pro | Ile | Asn | Glu | Asp | Leu | Asp | Glu | Gly | Ile | Met | Val | Val |
| | | 115 | | | | | 120 | | | | | 125 | | | |

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Tyr Lys Arg Asn Ile Val Ala His Thr Phe Lys Val Arg Val Tyr Gln
 130 135 140
 Lys Val Leu Thr Phe Arg Arg Ser Tyr Ala Tyr Ile His Thr Thr Tyr
 145 150 155 160
 Leu Leu Gly Ser Asn Thr Glu Tyr Val Ala Pro Pro Met Trp Glu Ile
 165 170 175
 His His Ile Asn Ser His Ser Gln Cys Tyr Ser Ser Tyr Ser Arg Val
 180 185 190
 Ile Ala Gly Thr Val Phe Val Ala Tyr His Arg Asp Ser Tyr Glu Asn
 195 200 205
 Lys Thr Met Gln Leu Met Pro Asp Asp Tyr Ser Asn Thr His Ser Thr
 210 215 220
 Arg Tyr Val Thr Val Lys Asp Gln Trp His Ser Arg Gly Ser Thr Trp
 225 230 235 240
 Leu Tyr Arg Glu Thr Cys Asn Leu Asn Cys Met Val Thr Ile Thr Thr
 245 250 255
 Ala Arg Ser Lys Tyr Pro Tyr His Phe Phe Ala Thr Ser Thr Gly Asp
 260 265 270
 Val Val Asp Ile Ser Pro Phe Tyr Asn Gly Thr Asn Arg Asn Ala Ser
 275 280 285
 Tyr Phe Gly Glu Asn Ala Asp Lys Phe Phe Ile Phe Pro Asn Tyr Thr
 290 295 300
 Ile Val Ser Asp Phe Gly Arg Pro Asn Ser Ala Leu Glu Thr His Arg
 305 310 315 320
 Leu Val Ala Phe Leu Glu Arg Ala Asp Ser Val Ile Ser Trp Asp Ile
 325 330 335
 Gln Asp Glu Lys Asn Val Thr Cys Gln Leu Thr Phe Trp Glu Ala Ser
 340 345 350
 Glu Arg Thr Ile Arg Ser Glu Ala Glu Asp Ser Tyr His Phe Ser Ser
 355 360 365
 Ala Lys Met Thr Ala Thr Phe Leu Ser Lys Lys Gln Glu Val Asn Met
 370 375 380
 Ser Asp Ser Ala Leu Asp Cys Val Arg Asp Glu Ala Ile Asn Lys Leu
 385 390 395 400

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Gln Gln Ile Phe Asn Thr Ser Tyr Asn Gln Thr Tyr Glu Lys Tyr Gly
 405 410 415
 Asn Val Ser Val Phe Glu Thr Thr Gly Gly Leu Val Val Phe Trp Gln
 420 425 430
 Gly Ile Lys Gln Lys Ser Leu Val Glu Leu Glu Arg Leu Ala Asn Arg
 435 440 445
 Ser Ser Leu Asn Leu Thr His Asn Arg Thr Lys Arg Ser Thr Asp Gly
 450 455 460
 Asn Asn Ala Thr His Leu Ser Asn Met Glu Ser Val His Asn Leu Val
 465 470 475 480
 Tyr Ala Gln Leu Gln Phe Thr Tyr Asp Thr Leu Arg Gly Tyr Ile Asn
 485 490 495
 Arg Ala Leu Ala Gln Ile Ala Glu Ala Trp Cys Val Asp Gln Arg Arg
 500 505 510
 Thr Leu Glu Val Phe Lys Glu Leu Ser Lys Ile Asn Pro Ser Ala Ile
 515 520 525
 Leu Ser Ala Ile Tyr Asn Lys Pro Ile Ala Ala Arg Phe Met Gly Asp
 530 535 540
 Val Leu Gly Leu Ala Ser Cys Val Thr Ile Asn Gln Thr Ser Val Lys
 545 550 555 560
 Val Leu Arg Asp Met Asn Val Lys Glu Ser Pro Gly Arg Cys Tyr Ser
 565 570 575
 Arg Pro Val Val Ile Phe Asn Phe Ala Asn Ser Ser Tyr Val Gln Tyr
 580 585 590
 Gly Gln Leu Gly Glu Asp Asn Glu Ile Leu Leu Gly Asn His Arg Thr
 595 600 605
 Glu Glu Cys Gln Leu Pro Ser Leu Lys Ile Phe Ile Ala Gly Asn Ser
 610 615 620
 Ala Tyr Glu Tyr Val Asp Tyr Leu Phe Lys Arg Met Ile Asp Leu Ser
 625 630 635 640
 Ser Ile Ser Thr Val Asp Ser Met Ile Ala Leu Asp Ile Asp Pro Leu
 645 650 655
 Glu Asn Thr Asp Phe Arg Val Leu Glu Leu Tyr Ser Gln Lys Glu Leu
 660 665 670

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| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Ser | Ser | Asn | Val | Phe | Asp | Leu | Glu | Glu | Ile | Met | Arg | Glu | Phe | Asn |
| | | 675 | | | | | 680 | | | | | 685 | | | |
| Ser | Tyr | Lys | Gln | Arg | Val | Lys | Tyr | Val | Glu | Asp | Lys | Val | Val | Asp | Pro |
| | 690 | | | | | 695 | | | | | 700 | | | | |
| Leu | Pro | Pro | Tyr | Leu | Lys | Gly | Leu | Asp | Asp | Leu | Met | Ser | Gly | Leu | Gly |
| 705 | | | | | 710 | | | | | 715 | | | | | 720 |
| Ala | Ala | Gly | Lys | Ala | Val | Gly | Val | Ala | Ile | Gly | Ala | Val | Gly | Gly | Ala |
| | | | | 725 | | | | | 730 | | | | | 735 | |
| Val | Ala | Ser | Val | Val | Glu | Gly | Val | Ala | Thr | Phe | Leu | Lys | Asn | Pro | Phe |
| | | | 740 | | | | | 745 | | | | | 750 | | |
| Gly | Ala | Phe | Thr | Ile | Ile | Leu | Val | Ala | Ile | Ala | Val | Val | Ile | Ile | Ile |
| | | 755 | | | | | 760 | | | | | 765 | | | |
| Tyr | Leu | Ile | Tyr | Thr | Arg | Gln | Arg | Arg | Leu | Cys | Met | Gln | Pro | Leu | Gln |
| | 770 | | | | | 775 | | | | | 780 | | | | |
| Asn | Leu | Phe | Pro | Tyr | Leu | Val | Ser | Ala | Asp | Gly | Thr | Thr | Val | Thr | Ser |
| 785 | | | | | 790 | | | | | 795 | | | | | 800 |
| Gly | Asn | Thr | Lys | Asp | Thr | Ser | Leu | Gln | Ala | Pro | Pro | Ser | Tyr | Glu | Glu |
| | | | | 805 | | | | | 810 | | | | | 815 | |
| Ser | Val | Tyr | Asn | Ser | Gly | Arg | Lys | Gly | Pro | Gly | Pro | Pro | Ser | Ser | Asp |
| | | | 820 | | | | | 825 | | | | | 830 | | |
| Ala | Ser | Thr | Ala | Ala | Pro | Pro | Tyr | Thr | Asn | Glu | Gln | Ala | Tyr | Gln | Met |
| | | 835 | | | | | 840 | | | | | 845 | | | |
| Leu | Leu | Ala | Leu | Val | Arg | Leu | Asp | Ala | Glu | Gln | Arg | Ala | Gln | Gln | Asn |
| | 850 | | | | | 855 | | | | | 860 | | | | |
| Gly | Thr | Asp | Ser | Leu | Asp | Gly | Gln | Thr | Gly | Thr | Gln | Asp | Lys | Gly | Gln |
| 865 | | | | | 870 | | | | | 875 | | | | | 880 |
| Lys | Pro | Asn | Leu | Leu | Asp | Arg | Leu | Arg | His | Arg | Lys | Asn | Gly | Tyr | Arg |
| | | | | 885 | | | | | 890 | | | | | 895 | |
| His | Leu | Lys | Asp | Ser | Asp | Glu | Glu | Glu | Asn | Val | | | | | |
| | | | 900 | | | | | 905 | | | | | | | |

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACACGCAAGA GATCTAGACG CGCCTCAT

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCGTCCAGAC TCTAGAGGTA GGGC

24

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGACTCCATT CTAGATTAAT GAGTTGCATT

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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CAAAGTCGGA GTCTAGAGTC TAGTTCGGAA A

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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAGATAAGTG GTCTAGATCT AAGCGTAGCT ACG

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WHAT IS CLAIMED IS:

1. A non-defective recombinant adenovirus containing a human cytomegalovirus protein gene encoding a cytomegalovirus protein gB subunit fragment containing at least one CTL epitope, said gene being under the control of an expression control sequence, said virus capable of expressing said subunit protein.

2. The adenovirus according to claim 1 wherein said fragment is selected from the group consisting of:

(a) the fragment spanning about amino acid 1 to about amino acid 303,

(b) the fragment spanning about amino acid 1 to about amino acid 700,

(c) the fragment spanning about amino acid 1 to about amino acid 465,

(d) fragments spanning about amino acid 155 to about amino acid 303, and

(e) smaller fragments of (a) through (d) of SEQ ID NO:2.

3. An immunogenic composition comprising a non-defective recombinant adenovirus and a suitable pharmaceutical carrier, wherein said recombinant adenovirus comprises a human cytomegalovirus protein gene encoding a cytomegalovirus protein gB subunit fragment containing at least one CTL epitope, said gene being under the control of an expression control sequence and said virus is capable of expressing said subunit protein *in vivo* in an animal.

4. The composition according to claim 3 wherein said fragment is selected from the group consisting of:

(a) the fragment spanning about amino acid 1 to about amino acid 303,

(b) the fragment spanning about amino acid 1 to about amino acid 700,

(c) the fragment spanning about amino acid 1 to about amino acid 465,

(d) fragments spanning about amino acid 155 to about amino acid 303, and

(e) smaller fragments of (a) through (d) of SEQ ID NO:2.

5. The composition according to claim 3 wherein said protein gene encodes an additional cytomegalovirus subunit protein fragment or a selected cytomegalovirus subunit protein.

6. The composition according to claim 3 wherein said gB subunit fragment is about amino acid 1 to about amino acid 303 of SEQ ID NO:2.

7. The composition according to claim 3 wherein said adenovirus is selected from the group consisting of an adenovirus type 5, adenovirus type 4 and adenovirus type 7 strain.

8. The composition according to claim 7 wherein said gB subunit fragment is obtained from the Towne strain cytomegalovirus, and the adenovirus is type 5.

9. The use of a non-defective recombinant adenovirus comprising a human cytomegalovirus protein gene encoding a cytomegalovirus protein gB subunit fragment containing at least one CTL epitope, said gene being under the control of an expression control sequence and said virus being capable of expressing said subunit protein *in vivo* in an animal, in the preparation of a CMV vaccine.

10. The use according to claim 9 wherein said fragment is selected from the group consisting of:

(a) the fragment spanning about amino acid 1 to about amino acid 303,

(b) the fragment spanning about amino acid 1 to about amino acid 700,

(c) the fragment spanning about amino acid 1 to about amino acid 465,

(d) fragments spanning about amino acid 155 to about amino acid 303, and

(e) smaller fragments of (a) through (d) of SEQ ID NO:2.

11. The use according to claim 9 wherein said adenovirus is present in an effective amount of between 10^5 to 10^8 plaque forming units.

12. An immunogenic composition comprising a gB subunit protein fragment containing at least one CTL epitope expressed in a recombinant adenovirus vector.

13. The composition according to claim 12 wherein said fragment is selected from the group consisting of:

(a) the fragment spanning about amino acid 1 to about amino acid 303,

(b) the fragment spanning about amino acid 1 to about amino acid 700,

(c) the fragment spanning about amino acid 1 to about amino acid 465,

(d) fragments spanning about amino acid 155 to about amino acid 303, and

(e) smaller fragments of (a) through (d) of SEQ ID NO:2.

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FIG. 1A

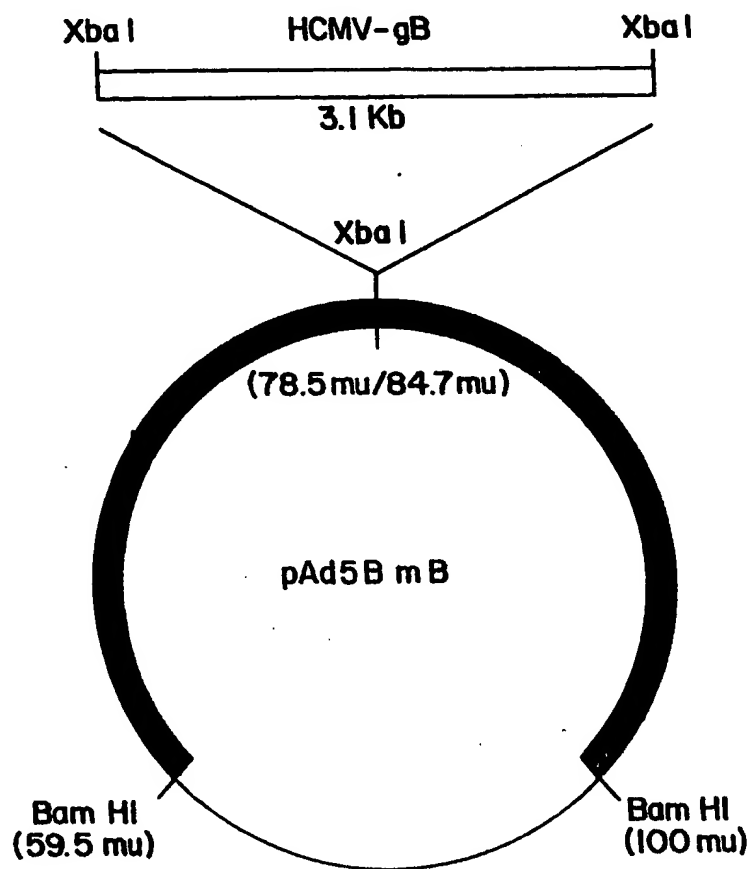
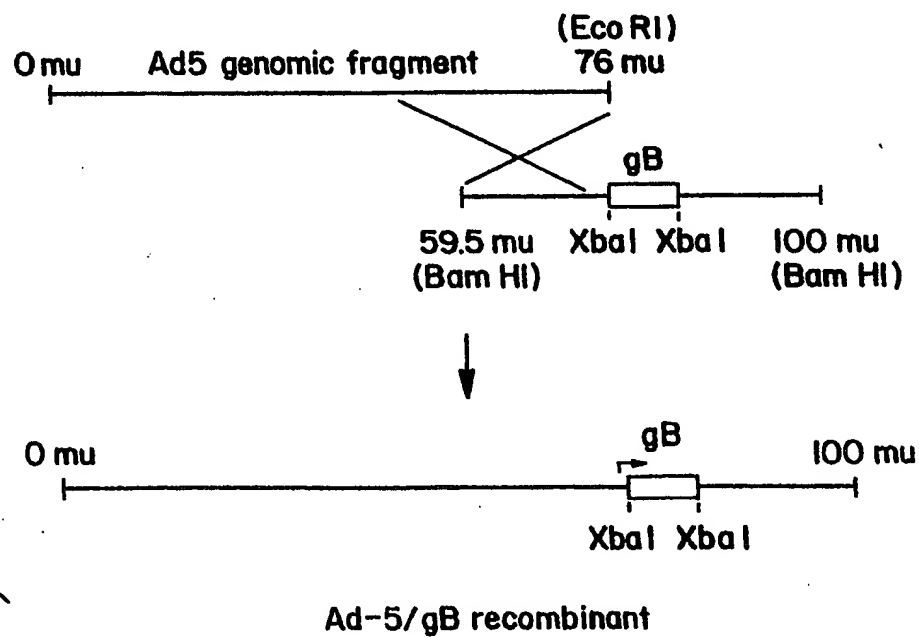


FIG. 1B



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/04180

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : Please See Extra Sheet.

US CL : 424/88, 89; 435/235.1, 69.3, 5, 69.1, 172.3; 436/543; 514/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/88, 89; 435/235.1, 69.3, 5, 69.1, 172.3; 436/543; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

INTELLIGENETICS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------|
| Y | THE EMBO JOURNAL, Volume 5, No. 11, issued November 1986, Cranage et al., "Identification Of The Human Cytomegalovirus Glycoprotein B Gene and Induction of Neutralizing Antibodies via Its Expression in Recombinant Vaccinia Virus", pages 3057-3063, see entire document. | 1-13 |
| Y | US, A, 4,920,209 (DAVIS ET AL.) 24 April 1990, see entire document. | 1-13 |



Further documents are listed in the continuation of Box C.



See patent family annex.

| | |
|---|--|
| * Special categories of cited documents: | * later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| *A* document defining the general state of the art which is not considered to be of particular relevance | *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| *E* earlier document published on or after the international filing date | *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | *Z* document member of the same patent family |
| *O* document referring to an oral disclosure, use, exhibition or other means | |
| *P* document published prior to the international filing date but later than the priority date claimed | |

Date of the actual completion of the international search

22 JULY 1994

Date of mailing of the international search report

02 AUG 1994

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Box PCT
Washington, D.C. 20231

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Authorized officer

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Telephone No. (703) 308-0196

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A61K 39/00, 39/12; C12N 7/00, 15/00; C12P 21/06; C12Q 1/70; G01N 33/531; A01N 43/04